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14. ABSTRACT

Patients with breast cancer can develop recurrent metastatic disease with latency periods that range from years to decades. This pause can be explained by micrometastatic disseminated tumor cell (DTC) dormancy, a stage in cancer progression in which residual disease is present but remains asymptomatic and is clinically undetectable. Cancer dormancy remains an outstanding challenge for both clinicians and scientists. Recent findings have suggested that the cancer dormancy signaling program is characterized by low ERK:p38 MAPK signaling ratio and prolonged G0/G1 arrest, reminiscent of cancer stem cells, and is thought to be a major mechanism of resistance to conventional cytotoxic chemotherapy. Cancer EMT, which is thought to precede cancer dormancy, may also contribute directly to the cancer dormancy program through its ability to induce low ERK:p38 signaling ratio. The central role of p38 in cancer dormancy was recently demonstrated by the ability of p38 inhibitors (e.g. SB203580 or dominant negatives) to break dormancy and induce tumor growth, making it an attractive therapeutic target.

Here we have proposed to test a novel therapeutic concept that breast cancer dormancy can be controlled by acutely forcing dormant cancer cells into rapid proliferation, thereby rendering them sensitive to killing by cytotoxic chemotherapy. We tested the feasibility of this innovative approach by inhibiting p38 MAPK before exposing cancer cells to cytotoxic chemotherapy. We used the MTB/TAN mouse model, a Tet-inducible activated human her2/Neu model, which produces PR/ER-negative and ErbB2-independent recurrent tumors (similar to human triple negative breast cancer) after withdrawal of doxycycline (deinduction). These recurrent tumors have all the characteristic of having undergone EMT and are relatively growth arrested and dormant. Using these mice and cells derived from these recurrent tumors, we would determine whether pretreatment with the specific and potent p38 inhibitor SB203580 will increase paclitaxel cytotoxicity in vitro and lead to decreased lung metastatic burden and increased overall survival in vivo in this mouse model of cancer EMT and dormancy. However, we were not able to reproduce the high penetrance of this deinduction model as reported in the literature. Alternatively we tested mice with ErbB2-positive tumors during the induction phase before doxycycline withdrawal and found this model to be closely mimicking human breast cancer metastasis and dormancy. We will use this model instead to test our hypothesis. Our proposed study could provide a proof of principle for this novel therapeutic concept and has the potential to change clinical practice not only in breast cancer but also in many other malignancies.

15. SUBJECT TERMS

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INTRODUCTION:

We proposed to test the hypothesis that tumor dormancy can be prevented by reactivating proliferation in dormant tumor cells before exposing them to cytotoxic chemotherapy. To accomplish this goal, we proposed to use the compound SB203580, a potent p38 inhibitor, to unravel the EMT-p38 signaling loop that is associated with growth arrest in dormant tumor cells and to reactivate cell proliferation before exposing dormant cells to chemotherapy.

BODY:

We proposed to use the MMTV-rtTA;TetO-NeuNT or MTB/TAN mouse model. It is a Tet-inducible activated Neu/ErbB2 model in FVB mice. As previously reported, multiple primary breast cancers develop with 100% penetrance at 6 weeks. Removal of doxycycline at this point leads to primary tumor regression and subsequent spontaneous local recurrences with 86% penetrance and a 16-week latency period[1]. These recurrent tumors do not express PR/ER and NeuNT (similar to human triple negative breast cancer), develop independently of doxycycline treatment, express high level of Snail1, and exhibit many cellular features of EMT and dormancy, including mesenchymal morphology, low ERK:p38 signaling ratio and much slower growth rates as evidenced by the much longer latency period (16 weeks vs. 6 weeks), and thus are considered to have initiated an EMT program. Lung metastases emerge 2-3 months later, although disseminated tumor cells (DTCs) can be detected in the bone marrow well in advance, indicating the presence of prolonged dormant tumor cells. Our original plan was to determine whether pretreatment of ErbB2-positive (prior to induction) and ErbB2-negative breast tumors (recurrent tumors) with SB203580 followed exposure to Taxol will increase taxol cytotoxicity (IC_{50}). Once that is established, we proposed to determine whether breaking cancer dormancy could increase tumor sensitivity to cytotoxic dugs *in vivo*.

For the first 6 months of the project, we expanded the MTB/TAN mouse colony significantly to obtain adequate numbers necessary for the proposed experiments. Nearly 100% of mice treated with doxycycline (induction) developed large breast tumors. Unfortunately, after doxycycline withdrawal (deinduction) we were not successful at reproducing the results reported in ref. 1. In fact, with a cohort of >30 deinduced mice, we were not able to obtain any recurrent tumors even at least 35 weeks after deinduction, even though the reported penetrance of recurrent tumors was supposed to be 86%.

As an alternative, we tested MTB/TAN mice with ErbB2-positive breast tumors (prior to deinduction) and determined whether the Snail1-Twist1 cooperation in breast cancer metastasis and dormancy is present, and whether dormant bone marrow DTCs (BM DTCs) can be detected using immunofluorescence and qRT-PCR for ErbB2, Snail1, and Twist1. In human breast cancer, high expression of Twist1 in BM DTCs is associated with increased p38 activation, prolonged growth arrest, and late recurrence[2].

If so, we can test the central hypothesis of this proposal (SB203580 pretreatment predisposes dormant tumor cells to increased sensitivity to cytotoxic agents) using ErbB2-positive tumors without having to wait for recurrent tumors. We compared and contrasted expression of Snail1, Twist1, and CK in both primary tumors and BM DTCs isolated from mice with and without overt lung metastasis within 3 months of palpable tumors. Consistent with earlier report[3], we detected BM DTCs by ErbB2 IF as soon as palpable tumors were present and well before overt metastases noted (Fig. 1), suggesting that dormant BM DTCs were present. Similar to human breast cancer, Snail1 expression in primary tumors correlated with metastasis (Fig. 2). In contrast, Twist1 expression was much more highly expressed in BM DTCs in mice that developed metastasis (Fig. 2). Thus, the Snail1-Twist1 temporal and spatial cooperation exists in these mice and correlates with distant relapses just as in human breast cancer, suggesting that they are a good model to test our hypothesis.

To determine whether pretreatment with the p38 inhibitor SB203580 prior to Taxol exposure would increase Taxol cytotoxicity in cells expressing high levels of Twist1 and thus being dormant, we established 2 independent lines of immortalized MCF10A cells overexpressing Twist1. We have shown earlier that these cell lines were growth arrested and expressed high levels of Twist1 and p38 activity, and that treatment of these cells with SB203580 was able to reactivate cellular proliferation[2]. As expected, pretreatment of these cells with SB203580 significantly increased Taxol cytotoxicity (from >40nM to $\leq$10nM) (Fig. 3), suggesting effective p38 inhibition is capable of breaking dormancy in vitro. We are in the process of establishing stable lines of ErbB2-positive breast cancer cells isolated from this mouse model to repeat a similar experiment. But first we are in the process of determining whether ErbB2-positive primary tumors have elevated p38 activity. These should be accomplished within the next month.

Once these are established, we will move forward with testing this concept in the ErbB2 models as detailed in the proposal in the next 3-4 months.

KEY RESEARCH ACCOMPLISHMENTS:

1) ErbB2-positive tumors express Snail1.
2) ErbB2 mice have detectable dormant BM DTCs well before detectable metastases.
3) The Snail1-Twist1 cooperation in breast cancer metastasis and dormancy is present in the ErbbB2-positive breast cancer model mice, similar to human breast cancer.
4) Pretreatment of Twist1-expressing, dormant cells with the p38 inhibitor SB203580 was able to break dormancy and increase chemotherapy cytotoxicity, at least in vitro. (In vivo data are pending)

REPORTABLE OUTCOMES:
Once the in vivo data is available and supports our hypothesis, we intend to submit a proposal for funding of a clinical trial testing this concept within the next year.

SUPPORTING DATA:

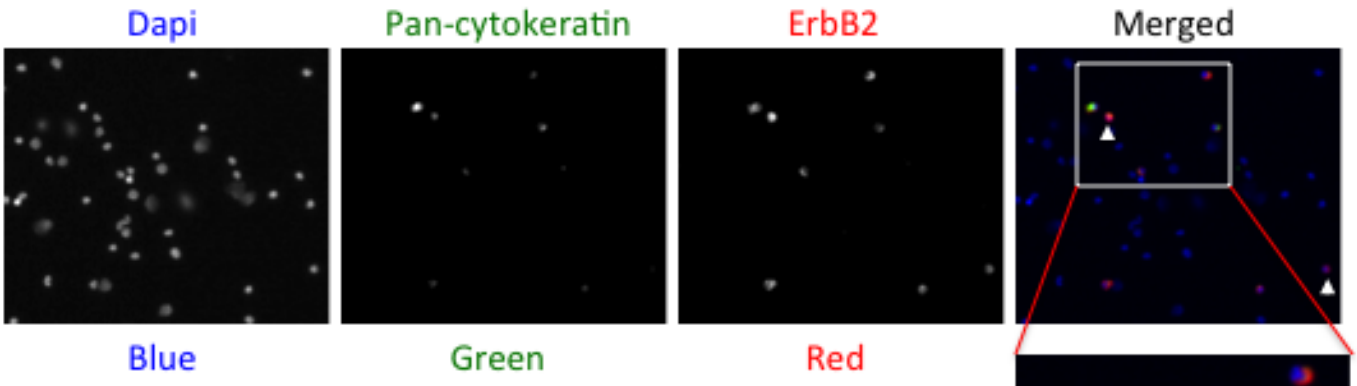


Figure 1: BM DTCs are present 1 month after resection of primary tumor in ErbB2 mice. Many DTCs are ErbB2-positive and CK-positive. Few are ErbB2-positive and CK-negative/low (arrowheads), suggesting that these are likely DTCs in EMT or partial EMT.

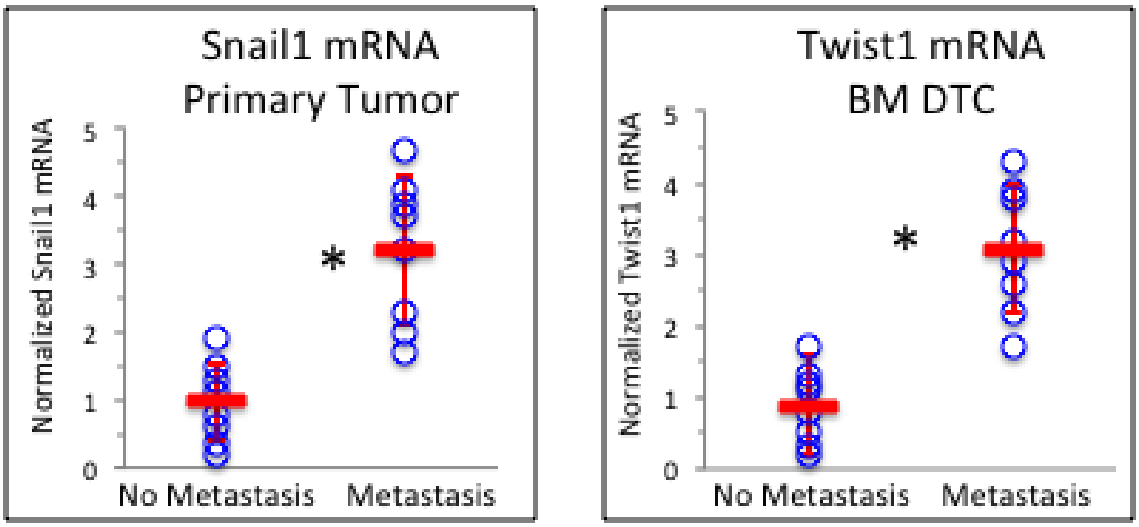
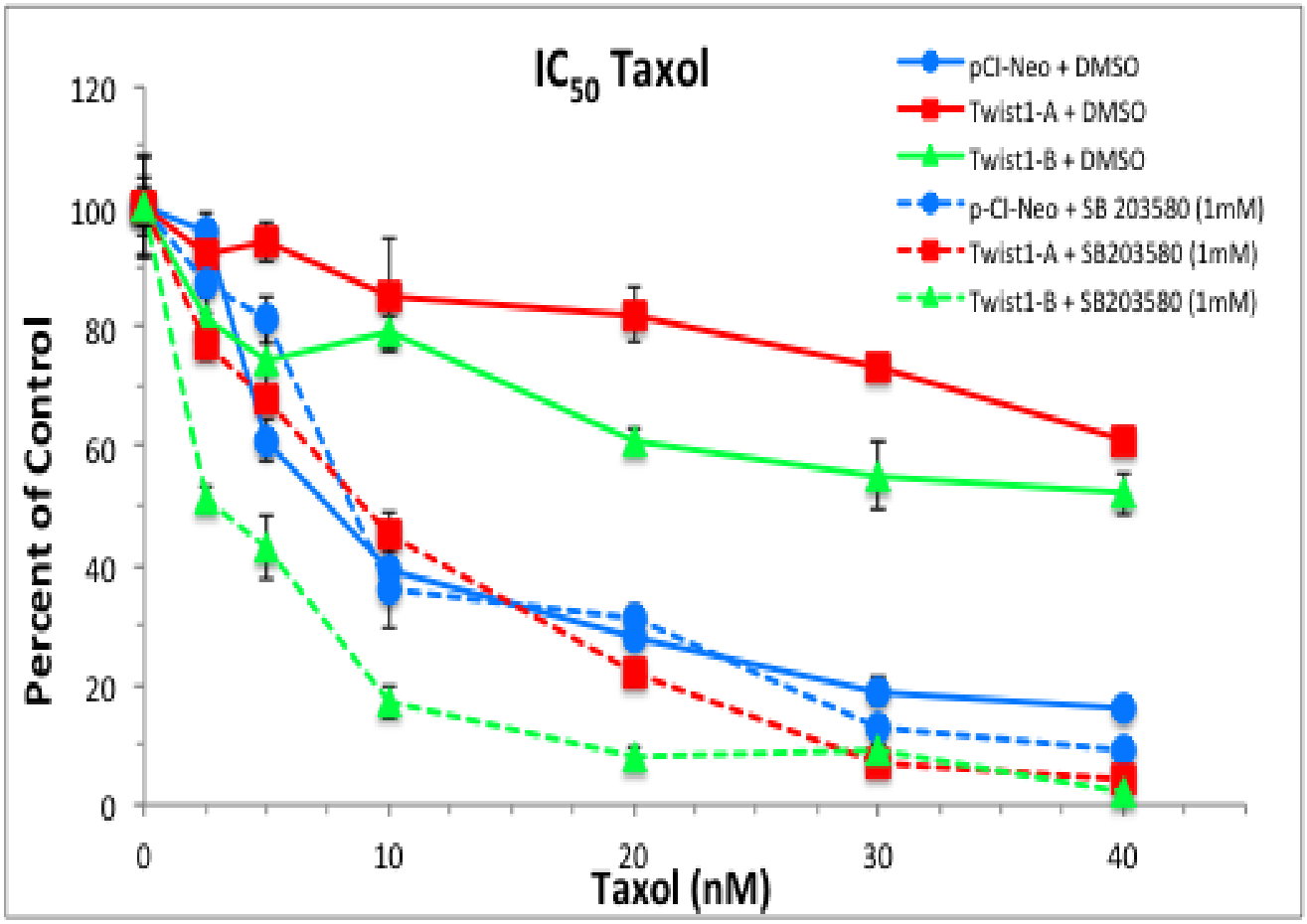


Figure 2: The Snail1-Twist1 cooperation is observed in ErbB2 mice. Snail1 and Twist1 mRNAs were measured and normalized against GAPDH in primary tumors and BM (*, $p<0.05$).



IC50 Taxol (nM)			
	pCI-Neo	Twist1-A	Twist1-B
DMSO	7.5	>40	>40
SB 203580 (1mM)	8.3	10	2.5

Figure 3: Pretreatment of Twist1-expressing, dormant MCF10A cells with the p38 inhibitor SB203580 breaks dormancy and increases Taxol cytotoxicity. Cells were treated with 1mM SB203580 72hrs prior, during, and 96hrs after exposure to increasing concentrations of Taxol. Numbers of remaining live cells were determined and expressed as percentages of no Taxol control.

CONCLUSION:

We have shown that the ErbB2-positive breast tumor model recapitulates the Snail1-Twist cooperation in human breast cancer metastasis and dormancy. We have evidence in Twist1-expressing, dormant immortalized MCF10A cells that dormancy can be reversed by effective inhibition of p38 MAPK activity, which results in dormant cells reentering the cell cycle making them more sensitive to cytotoxic chemotherapy. Current experiments are aimed at confirming these results in ErbB2 mice before moving forward with clinical trial in human patients.

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APPENDICES: N/A